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Biological Control 28 (2003) 129–136
Biological Control
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Comparison of beneficial traits among strains of the entomopathogenic nematode, *Steinernema carpocapsae*, for control of *Curculio caryae* (Coleoptera: Curculionidae)

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Received 6 November 2002; accepted 23 January 2003

Abstract

Our objective was to compare beneficial traits among strains of *Steinernema carpocapsae* to identify or develop a superior biocontrol candidate for suppression of the pecan weevil, *Curculio caryae*. Virulence, environmental tolerance to heat and desiccation, and reproductive capacity were compared among eight strains. Fitness (in vitro growth) of the symbiotic bacteria, *Xenorhabdus nematophila* isolated from six of the nematode strains, was also compared. Significant differences were detected among nematode and bacteria strains for each trait. All nematode strains were more virulent to *C. caryae* adults than to larvae. No single *S. carpocapsae* strain was superior for all beneficial traits measured. Overall, Breton, DD136, Italian, and Kapow strains were ranked inferior to other strains. Agriotos, All, and Sal strains were superior when desiccation was a factor. When desiccation tolerance was removed as a factor, the Mexican strain also tended to fall into the superior rankings.

Keywords: Beneficial traits; Biological control; *Curculio caryae*; Pecan weevil; *Steinernema carpocapsae*; *Xenorhabdus nematophila*

1. Introduction

The pecan weevil, *Curculio caryae* (Horn), is a key pest of pecan throughout the Southeastern US as well as in portions of Texas and Oklahoma (Payne and Dutcher, 1985). The insects have a 2 or 3 year life cycle (Harris, 1985). Adults emerge from soil in late July–August and feed on and oviposit in nuts (Harris, 1985). Larvae develop within the nut and fourth instars drop to the ground where they burrow to a depth of 8–25 cm, form a soil cell, and over-winter. The following year approximately 90% of the larvae pupate and spend the next 9 months in the soil as adults (Harris, 1985). The remaining 10% of the population spend 2 years in the soil as larvae and emerge as adults in the third year (Harris, 1985).

Control recommendations for *C. caryae* currently consist solely of foliar applications of chemical insecticides (e.g., carbaryl) to kill adults (Harris, 1999). Due to the problems associated with aphid resurgence (Dutcher and Payne, 1985), as well as other environmental and regulatory concerns, research on developing alternative control strategies is warranted. Entomopathogenic nematodes are one of the potential alternatives.

Entomopathogenic nematodes are obligate parasites in the genera *Steinernema* and *Heterorhabditis*. Entomopathogenic nematodes kill insects with the aid of a mutualistic bacterium, which is carried in their intestine (Poinar, 1990). The nematodes complete 2–3 generations within the host, after which free-living infective juveniles (IJs) emerge to seek new hosts (Poinar, 1990). Entomopathogenic nematodes are effective at controlling a variety of economically important pests including the larvae of several weevil species (Coleoptera: Curculionidae) such as the black vine weevil, *Otiorhynchus sulcatus* (F.), and the Diaprepes root weevil, *Diaprepes abbreviatus* (L.) (Shapiro-Ilan et al., 2002a).

The potential for control of *C. caryae* larvae with entomopathogenic nematodes appears to be poor

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doi:10.1016/S1049-9644(03)00030-6

whereas promise for control of adults is substantial (Shapiro-Ilan, 2001a,b). Field trials to suppress *C. caryae* larvae with *Heterorhabditis bacteriophora* (Poinar), *Steinernema carpocapsae* (Weiser), or *Steinernema feltiae* (Filipjev) have resulted in less than 35% control (Nyczepir et al., 1992; Smith et al., 1993). Furthermore, in a laboratory study, Shapiro-Ilan (2001a) found the virulence of 15 strains from nine species of entomopathogenic nematodes to be poor against larvae. Contrarily, when adult weevils were tested under identical conditions, several nematode species caused high levels of mortality (Shapiro-Ilan, 2001b). *S. carpocapsae* (All strain) in particular was found to have a high level of virulence to adult *C. caryae* causing 99% control 4 days after treatment (Shapiro-Ilan, 2001b).

Successful biological control with entomopathogens (such as steinernematid and heterorhabditid nematodes) can depend on possession of certain beneficial traits (Fuxa, 1987; Gaugler, 1987). In entomopathogenic nematodes, various beneficial traits have been reported to differ among strains within a species, e.g., virulence (Morris and Converse, 1991; Shapiro and McCoy, 2000), reproductive potential (Somasekhar et al., 2002), and environmental tolerance (Glazer, 2002; Shapiro et al., 1996; Somasekhar et al., 2002). Our objective was to compare beneficial traits among strains of *S. carpocapsae*. We reasoned that if one strain is superior to others in most or all of the beneficial traits, then it would likely be a superior candidate for biocontrol of *C. caryae*. Additionally, obtaining knowledge related to the diversity and distribution of beneficial traits may facilitate genetic improvement (Gaugler, 1987; Glazer et al., 1991; Somasekhar et al., 2002).

Somasekhar et al. (2002) compared virulence, reproductive capacity, and environmental tolerance (i.e., tolerance to heat, desiccation, and hypoxia) among 14 populations of *S. carpocapsae*, and found substantial variation in these traits among them. Our research differed from that of Somasekhar et al. (2002) in several aspects. Their research compared freshly isolated natural populations of *S. carpocapsae*. In contrast, we studied variation in beneficial traits among seven strains (Agriotos, All, Breton, DD136, Italian, Kapow, and Mexican) that have been in culture for over 15 years (Agudelo-Silva et al., 1987; Poinar, 1986), and one (Sal) that has been in culture at least 5 years (K. Nguyen, personal communication). Beneficial traits in entomopathogenic nematodes are known to change or deteriorate over time in laboratory culture (Shapiro et al., 1996; Stuart and Gaugler, 1996; Wang and Grewal, 2002). We used strains that already had been cultured for numerous generations because the beneficial traits in these strains are likely to be more fixed (i.e., less likely to change) during subsequent sub-culturing than freshly isolated ones (Shapiro et al., 1996). Additionally, our study diverged from that of Somasekhar et al. (2002) in that our virulence tests targeted a specific pest (*C. caryae*), whereas their virulence tests used the factitious host, *Galleria mellonella* (L.). We chose an economic pest of interest because superior virulence to *G. mellonella* or any other host would not necessarily indicate superior virulence to the target pest.

2. Materials and methods

2.1. Nematodes and insects

Strains of *S. carpocapsae* (Agriotos, All, Breton, DD136, Italian, Kapow, Mexican, and Sal strains) were obtained from K. Nguyen (University of Florida). All nematodes were cultured in last instar *G. mellonella* according to procedures described in Kaya and Stock (1997). Nematodes were stored at 13 °C for up to 3 weeks prior to use. All nematode strains were included in all experiments except the Sal and Breton strains, which were omitted from the bacterial tests (because of difficulty isolating pure bacterial cultures in parallel with the other strains). *C. caryae* were obtained from an orchard in the USDA-ARS Research Farm, Byron, Georgia. Larvae (fourth instar) were collected from nuts and stored up to 3 months in sterile (autoclaved) soil at 4–10 °C prior to experimentation; adults (newly emerged) were collected in trunk traps and used within 3 days of capture (Shapiro-Ilan, 2001a,b).

2.2. Virulence and reproduction

Although prior studies indicated poor suitability of entomopathogenic nematodes (including *S. carpocapsae* All strain) for control of *C. caryae* larvae, we compared larval susceptibility to *S. carpocapsae* All strain with strains not tested previously to determine if an exception may exist. Virulence tests were conducted based on procedures described by Shapiro-Ilan (2001a). Experiments were conducted in plastic cups (Bioserv, Frenchtown, NJ) (3–4 cm in diameter, 3.5 cm deep) filled with (oven-dried) soil from the USDA-ARS pecan orchard (Byron, GA), and containing one larva each. The soil was a loamy sand with the percentage sand:silt:clay = 84:10:6, pH 6.1, and organic matter = 2.8% by weight. Approximately 500 IJs ($40/cm^2$) were pipetted onto the soil surface of each cup in 0.5 ml of water so that the final moisture was standardized at field capacity (14%). After inoculation, cups were incubated at 25 °C. Larval mortality was recorded 7 and 14 days post-inoculation. The experiment included an untreated control (only water added), was arranged in completely randomized design with four replicates of 10 cups for each treatment, and was repeated once.

Virulence of the various *S. carpocapsae* strains was also tested using adult *C. caryae*. The procedures were

identical to those described for the larvae except that a slice of apple (ca. 1 ± 0.4 g) was provided in each cup as food and a moisture source for the weevil (Shapiro-Ilan, 2001b). There were three replicates of 10 cups per treatment. Because of the greater susceptibility of adults relative to larvae, the rate of application was reduced (based on preliminary experimentation) to 100 IJs/cup ($8/cm^2$), and host mortality determined 24 and 72 h post-inoculation.

Nematode reproductive capacity was measured in *C. caryae* adults based on procedures described by Shapiro-Ilan (2001b). For each nematode strain, five infected *C. caryae* were placed on White traps (Kaya and Stock, 1997), from which IJs were collected until emergence ceased or was considered negligible (after 30 days post-inoculation). The number of IJs produced per insect was determined through dilution counts, and because the insect size may be correlated to number of nematodes produced (Flanders et al., 1996), the number of nematodes produced per gram of insect was also determined. The experiment was repeated once. Nematode reproductive capacity was not determined in *C. caryae* larvae because virulence levels were relatively low for all strains.

2.3. Environmental tolerance

Heat tolerance was measured using procedures described by Shapiro et al. (1996). Approximately 2000 IJs in 0.2 ml were pipetted into 5 ml of tap water in a 20-ml glass scintillation vial. The vial had already been equilibrated to 40 °C prior to addition of nematodes. After incubation for 2 h in a water bath shaker (rpm 70) at 40 °C, 0.2 ml of the suspension was transferred to a 60-mm petri dish containing 9 ml tap water. The dishes were incubated at 25 °C for 24 h at which time the percentage nematode mortality was determined based on movement response when probed with a dissecting needle. Treatments (strain) were replicated three times in a completely randomized design. The experiment was repeated once.

Desiccation tolerance was compared among nematode strains based on procedures described by Solomon et al. (1999). Approximately 2000 IJs were pipetted onto filter paper (55 mm, Whatman No. 1) in a 60-mm petri dish. Excess moisture was removed through vacuum filtration. The filter paper containing nematodes was then placed in a plastic desiccator (23 cm maximum diameter × 24-cm height, Nalgene, Rochester, NY) that was set to 85% RH based on a saturated solution of KCl. After 72 h of incubation at 25 °C the filter paper was removed and placed in approximately 5 ml tap water for an additional 24 h at which time percentage nematode mortality was determined using procedures described above. Each treatment (strain) contained three replicates in a randomized block design (blocked by desiccator); the experiment was repeated once.

2.4. Bacterial growth

Isolation of *Xenorhabdus nematophila* (Poinar and Thomas) from the hemolymph of *S. carpocapsae*-infected *G. mellonella*, verification of primary variant status using selective media, and subsequent culturing were based on procedures described by Akhurst (1980) and Kaya and Stock (1997). Bacteria used in experiments were grown in TSY [Tryptic Soy Broth (Difco, Detroit, MI) + 0.5% yeast extract (Sigma, St. Louis, MO)]. Approximately 8800 cells (started in TSY 24 h previously), in 1 ml was added to 50 ml of fresh TSY in a 300-ml Erlenmeyer flask. Flasks were placed on a rotary incubator shaker (Innova 4230, New Brunswick Scientific, Edison, NJ) at 25 °C and 250 rpm for 12 and 16 h at which time the density of bacteria was determined based on optical density (Dunphy and Webster, 1989; Jeffke et al., 2000) at 600 nm (Smart Spec 3000, Bio-Rad Laboratories, Hercules, CA). The experiment contained four replicate flasks for each treatment (bacteria isolate), was organized in a completely randomized design, and repeated twice.

2.5. Qualitative analysis of beneficial traits among strains

To facilitate comparison among strains, the performance of each strain for each beneficial trait was scored as follows. The strain was scored as 1 if performance was not significantly different from the highest level for that trait, −1 if performance was not significantly different from the lowest level for that trait, and 0 if performance was between the highest and lowest, or not significantly different from either. The scores among traits were then added for each strain. This comparison among traits was only done for control of *C. caryae* adults because virulence levels to larvae were relatively low for all strains. Bacterial growth was not included in the comparison.

2.6. Data analysis

In all experiments, treatment effects were determined through analysis of variance (Proc GLM), and if the ANOVA was significant, treatment differences were further elucidated through the LSD test ($\alpha = 0.05$) (SAS, 2001; Steel and Torrie, 1980). Prior to analysis, percentage data were arcsine of square root transformed; reproduction counts and spectrophotometer readings were square root transformed (Steel and Torrie, 1980).

3. Results

3.1. Virulence and reproduction

Although average *C. caryae* larval mortality from all *S. carpocapsae* strains was greater than the control, none

exceeded 50% ($F = 6.27$, df $= 8, 60$, $P = 0.0001$ for 7 days after treatment, and $F = 7.81$, df $= 8, 64$, $P = 0.0001$ for 14 days after treatment). Few differences in virulence were detected among strains; however, Italian strain caused lower mortality than three or four other strains 7 and 14 days after treatment (Fig. 1).

A number of differences in virulence to *C. caryae* adults were detected among *S. carpocapsae* strains (Fig. 2). The Italian strain caused greater adult mortality than four other strains (Agriotos, Breton, DD136, and Sal) 1 day after treatment ($F = 2.76$, df $= 8, 44$, $P = 0.015$). Three days after treatment the Mexican and Sal strain caused greater mortality than three other strains (Breton, DD136, and Kapow), and Italian and All strain were more virulent than Breton and DD136 ($F = 36.2$, df $= 8, 44$, $P = 0.0001$). All the strains caused greater mortality than the control (Fig. 2).

The number of IJs produced in *C. caryae* adults by the All strain was greater than the number produced by Italian, Breton, DD136, Kapow, and Mexican strains, and Agriotos and Sal strains produced more than Kapow ($F = 2.53$, df $= 7, 72$, $P = 0.022$) (Fig. 3). Analysis of reproductive capacity based on IJs per gram of insect did not provide any further resolution of treatment differences; the LSD separations were identical ($F = 3.21$, df $= 7, 72$, $P = 0.0052$).

3.2. Environmental tolerance

Heat tolerance at 40 °C was greater in *S. carpocapsae* DD136, Mexican, and Sal strains than All, Kapow, and Italian strains ($F = 3.0$, df $= 7, 40$, $P = 0.013$) (Fig. 4). Desiccation tolerance at 85% RH was poorest in Italian followed by Mexican and Sal strains ($F = 12.43$, df $= 7, 40$, $P = 0.0001$) (Fig. 4).

3.3. Bacterial growth

In vitro bacterial growth of the *S. carpocapsae* Italian strain 12 h after inoculation was more rapid than for other strains tested except Kapow ($F = 3.57$, df $= 5, 63$, $P = 0.0066$) (Fig. 5). By 16 h post-inoculation, no dif-

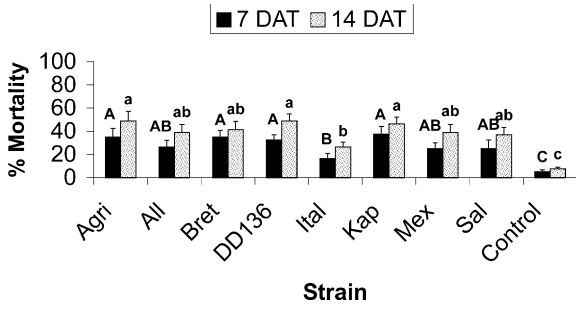

Fig. 1. Mean percentage mortality (±SEM) of *C. caryae* larvae 7 or 14 days after treatment (DAT) with various *S. carpocapsae* strains (Agriotis, All, Breton, DD136, Italian, Kapow, Mexican, Sal, or a water control). Different upper and lower case letters above bars indicate statistical differences 7 and 14 DAT, respectively (based on LSD tests).

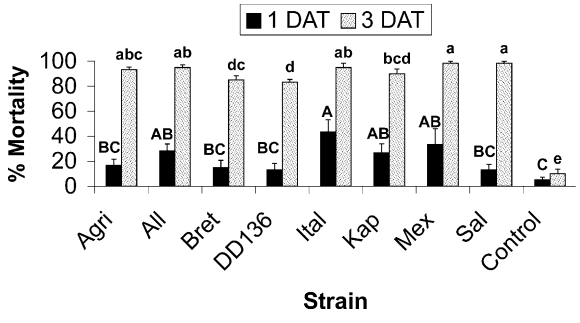

Fig. 2. Mean percentage mortality (±SEM) of *C. caryae* adults 1 or 3 days after treatment (DAT) with various *S. carpocapsae* strains (Agriotis, All, Breton, DD136, Italian, Kapow, Mexican, Sal, or a water control). Different upper and lower case letters above bars indicate statistical differences 1 and 3 DAT, respectively (based on LSD tests).

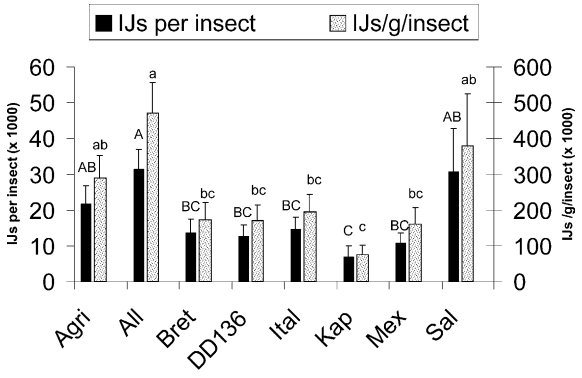

Fig. 3. Mean (±SEM) number of infective juvenile nematodes (IJs) produced per *C. caryae* adult, or per gram insect, with various *S. carpocapsae* strains (Agriotis, All, Breton, DD136, Italian, Kapow, Mexican, and Sal). Different upper and lower case letters above bars indicate statistical differences for IJs per insect and IJs/g/insect, respectively (based on LSD tests).

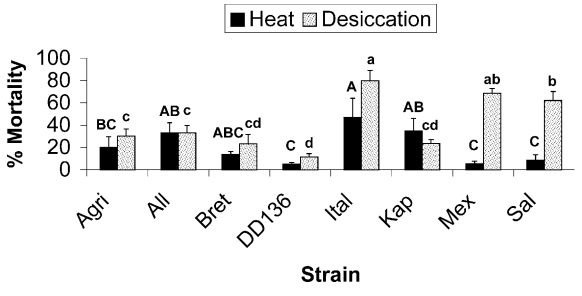

Fig. 4. Mean percentage mortality (±SEM) in various *S. carpocapsae* strains (Agriotis, All, Breton, DD136, Italian, Kapow, Mexican, and Sal) following exposure to 40 °C for 2 h (Heat), or 85% RH for 72 h (Desiccation). Different upper and lower case letters above bars indicate statistical differences for heat and desiccation tolerance, respectively (based on LSD tests).

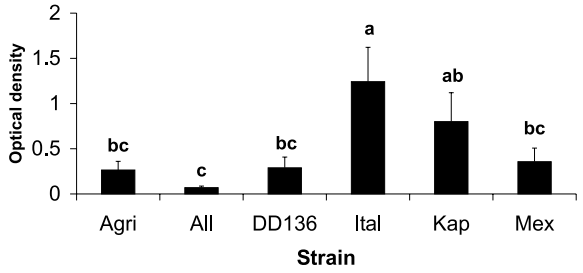

Fig. 5. In vitro growth of *X. nematophila* isolated from various *S. carpocapsae* strains (Agriotis, All, DD136, Italian, Kapow, and Mexican). Growth was measured by mean optical density (±SEM) at 600 nm following 12 h of shaking at 250 rpm and 25 °C. Different letters above bars indicate statistical differences (based on LSD tests).

ferences in bacterial growth were detected among strains ($F = 1.21$, df $= 7, 40$, $P = 0.315$) (data not shown).

3.4. Qualitative analysis of beneficial traits among strains

When performance scores among traits were added up for each *S. carpocapsae* strain and desiccation tolerance was included, Agriotos, All, and Sal strains scored among the highest, and Breton, DD136, Kapow, and Italian were among the lowest (Table 1). When desiccation tolerance was excluded, Sal strain and Agriotos scored the highest, All and Mexican were the second highest scoring, and Breton, DD136, and Kapow were among the lowest (Table 1).

4. Discussion

Laboratory screening of entomopathogenic nematodes for various beneficial traits has been used to identify superior candidates for insect suppression and has reduced the number of strains or species that need to be tested in the field (Mannion and Jansson, 1992; Patterson Stark and Lacey, 1999; Shapiro and McCoy, 2000). Our experiments did not distinguish any single *S. carpocapsae* strain to be superior to the others for all beneficial traits tested. Thus, it is difficult to predict which strain might be the most effective biological control agent for suppression of *C. caryae*. However, some strains exhibited superior performance in more traits than others. Therefore, some degree of selectivity might be in order; e.g., based on overall poor performance among traits, Breton, DD136, and Kapow strains may be less likely to be effective relative to other strains. Furthermore, our results indicate that under conditions where desiccation tolerance is required, Agriotos, All, and Sal strains may be the best choice among strains tested. Additionally, the Mexican strain may be among the better biocontrol candidates under conditions where the level of irrigation precludes a need for desiccation tolerance. Of course, field trials would be required to validate predictions made based on laboratory results.

In our qualitative comparison of beneficial traits among strains, we assumed all traits to have equal value in contributing to successful biological control of *C. caryae*. Some beneficial traits, however, are likely to be more important than others. Defining the precise contribution of each trait to pest control efficacy would be extremely difficult. On the other hand, some generalizations on the importance of each trait to *C. caryae* control can be made. A rapid ability to kill (like that exhibited by the Italian strain) may be especially important for control of *C. caryae* adults to prevent feeding and oviposition in the nut, which can occur as early as 2 days after the insects emerge (Criswell et al., 1975). Heat tolerance could be quite important because temperatures under the tree canopy (where nematodes would be applied) can reach levels that are detrimental to infectivity of *S. carpocapsae* (see Grewal et al., 1994). In Byron, Georgia, for example, temperatures in the orchard greater than 32 °C at or within 1 cm of the soil surface have been recorded (Shapiro-Ilan, unpublished).

Table 1
Qualitative comparison of beneficial traits among *S. carpocapsae* strains for adult *C. caryae* control[a]

Strain	Virulence 1 DAT[b]	Virulence 3 DAT	Reproductive capacity	Heat tolerance	Desiccation tolerance	Total with desiccation	Total without desiccation
Agriotos	−1	1	1	1	1	3	2
All	0	1	1	−1	1	2	1
Breton	−1	−1	−1	0	1	−2	−3
DD136	−1	−1	−1	1	1	−1	−2
Italian	1	1	−1	−1	−1	−1	0
Kapow	0	−1	−1	−1	1	−2	−3
Mexican	0	1	−1	1	−1	0	1
Sal	−1	1	1	1	0	2	2

[a] Rankings based on 1, performance equal to or not significantly different from the highest level achieved for that trait; −1, performance equal to or not significantly different from the lowest level of performance for that trait; 0, performance that is between the highest and lowest, or not significantly different from either (statistical groupings derived from LSD tests $\alpha = 0.05$). Totals represent sums of the scores for each trait within a strain.

[b] Days after treatment (DAT).

Because soil moisture is essential for nematode efficacy (Georgis and Gaugler, 1991; Kaya, 1990), desiccation tolerance may be an important factor, but only in orchards where irrigation is poorly or infrequently applied, and rainfall is insufficient.

Nematode reproductive capacity may be important in determining the potential for recycling. It is conceivable that *S. carpocapsae* strains producing approximately 30,000 IJs/*C. caryae* adult (such as All) could provide some level of recycling. Approximately 400 infected *C. caryae* would be required to duplicate a standard rate of $250,000\,\mathrm{IJs/m^2}$ (Georgis and Gaugler, 1991) applied to a $50\,\mathrm{m^2}$ area under a pecan tree canopy (where *C. caryae* emerges from). It is conceivable that this many infected *C. caryae* could result from prior nematode applications because weevil infestations can be more than 1000/tree (Cocke et al., 1984). The bulk of *C. caryae* adults emerge over a 4- to 6-week period (Harris, 1976); thus strains for which control levels persist longer (due to longevity or recycling) may reduce the need for multiple applications.

Although the nematode's symbiotic bacteria can be considered the key to killing the host (Forst and Clarke, 2002), they are most often ignored when screening is conducted to find the most likely biocontrol candidate. Different bacterial strains or species have been reported to differ in virulence (Dowds and Peters, 2002; Yeh and Alm, 1992). However, the most virulent bacterium to a given host is not necessarily associated with the most virulent nematode–bacterium complex (Yeh and Alm, 1992). Furthermore, it can be difficult to assign the relative contribution of virulence to either organism because the combination may result in synergy (Dowds and Peters, 2002). We found differences in in vitro growth rates among bacteria associated with different *S. carpocapsae* strains. The relationship between these differences and biocontrol potential is not known (hence bacterial growth was omitted from the qualitative comparison of traits). Indeed, it is not even clear whether superior growth in vitro will correlate well with growth in vivo (where host defenses must be overcome, see Dunphy and Thurston, 1990). It is, however, interesting to note that the Italian strain, which was most successful in rapidly killing the host (1-day post treatment), also had the fastest growing bacterium. Wright (1992) reported that entomopathogenic nematode reproductive rate was related to the symbiont's growth rate. We did not observe such a trend; nematodes with the highest reproduction (e.g., All and Agriotos strains) did not have bacteria with the highest growth rates. Now that it has been established that the *S. carpocapsae* bacterial strains vary in one character, it is reasonable to assume other differences exist. Further research is required to define the relationship between various bacterial strain differences and biocontrol potential of the nematode–bacteria complex.

Due to genetic processes such as inbreeding, drift, and inadvertent selection, one may expect genetic variation to decrease over time in biocontrol agents that are reared continuously under laboratory conditions (Hoy, 1986; Roush, 1990). Nevertheless, we detected significant variation in virulence, reproductive capacity, and environmental tolerance among *S. carpocapsae* strains that had been cultured in the laboratory for relatively long periods; the same traits in which Somasekhar et al. (2002) detected differences when comparing freshly isolated *S. carpocapsae* strains. Other studies found differences in virulence when comparing some of the same *S. carpocapsae* strains we included (Jackson and Brooks, 1989; Mannion and Jansson, 1992). Although our experiments did not lead us to choose a single superior strain, the diversity of traits that we did detect may lead to development of a superior strain through genetic improvement (Glazer et al., 1991; Somasekhar et al., 2002).

Genetic improvement may be accomplished through artificial selection (Gaugler et al., 1989) or hybridization (Shapiro et al., 1997). For example, an attractive combination would be the virulence of the Italian strain with the environmental tolerance of DD136. Recombining nematodes strains with each other's bacteria may also lead to improved biocontrol potential (Gerritsen et al., 1998); this would be all the more reason to characterize bacterial differences further.

This study confirms previous results indicating that the adult stage of *C. caryae* is more susceptible to entomopathogenic nematodes than the larval stage (Shapiro-Ilan, 2001a,b). Indeed, none of the *S. carpocapsae* strains tested in this study were more virulent to *C. caryae* larvae than All strain, which was previously reported to exhibit only poor to moderate virulence (Shapiro-Ilan, 2001b). Although a direct comparison could not be made (the insect stages are not available simultaneously), it is clear that nematode virulence to the adults is superior. Using essentially the same assay procedures, nematode induced mortality in adults after 3 days was close to double the mortality in larvae after 7 or 14 days despite a fivefold difference in application rate. Contrarily, in several weevil species, the larval stage is more susceptible to entomopathogenic nematode infection than the adult stage (Giblin-Davis et al., 1996; Mannion and Jansson, 1992; Morse and Lindegren, 1996; Pena et al., 1991). Larvae have also been observed to be more susceptible in other Coleoptera families (Geden et al., 1985; Georgis et al., 1991; Theunis, 1998). Recently in plum curculio, *Conotrachelus nenuphar*, (Herbst), Shapiro-Ilan et al. (2002b) observed *S. carpocapsae* to be relatively more virulent to adults, whereas *S. feltiae* was more virulent to larvae. Similarly, in this study, the relative virulence of some strains (e.g., Italian) varied according to insect stage.

An opportune period to apply entomopathogens for *C. caryae* control is when the insects are emerging from soil (Gottwald and Tedders, 1983; Shapiro-Ilan, unpublished). *S. carpocapsae* may be an ideal candidate for this approach because of it's ambusher host-seeking strategy (Lewis, 2002; Lewis et al., 1992) and tendency to remain near the surface of soil when applied there (Campbell et al., 1996; Moyle and Kaya, 1981). This behavior may be highly effective in infecting *C. caryae* as they emerge and crawl to the trunk. However, in field trials thus far, only approximately 60% suppression of adult *C. caryae* has been observed using *S. carpocapsae* All strain (Shapiro-Ilan, unpublished). In contrast, applications of carbaryl routinely result in over 95% suppression (Payne and Dutcher, 1985). Perhaps, with improved strains the level of suppression using *S. carpocapsae* can be increased.

Acknowledgments

We thank Lekeisha Buchanan, Wanda Evans, Shayna James, Kathy Halat, and Grace Lathrop for technical assistance, Dr. Khuong Nguyen for supplying nematode strains, and the Georgia Agricultural Commodity Commission for Pecan for funding a portion of this research.

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